REMARKS

The Examiner is thanked for the due consideration given the application. The specification has been amended to insert headings. A verified translation of priority document 02/12606 is attached to this paper. Also appended to this document are MADRI & WILLIAMS (J. Cell Biology), ATCC Search (Document 1) and ATCC Search (Document 2).

Claim 36 is pending in the application. Claims 1-35 and 37-48 have been canceled.

Amended claim 36 now recites a process for preparing a monoclonal antibody directed against endothelial cells with an angiogenic phenotype. This new subject matter corresponds to the subject matter of old claim 34.

Claim 36 has also been amended to recite that the antibody has the properties to bind to the surface of endothelial cells with angiogenic phenotype, and to specifically recognize said endothelial cells with angiogenic phenotype. This subject matter is suppported in the specification page 8, lines 11-16.

Also, amended claim 36 sets forth that the endothelial cells with an angiogenic phenotype has some specific characteristics regarding VEGF growth factor and VEGFR-2 receptor. This subject matter corresponds to canceled claim 31. The properties regarding VEGFR-2 is supported in the Example section, at page 27, lines 32-33 to page 28, line 1 of the specification.

Finally, claim 36 has been amended to recite that verification is made to test if the antibody inhibits the above-mentioned properties of cells with angiogenic properties.

No new matter is believed to be added to the application by this amendment.

Election/Restriction

Claims 27-35 and 37-48 have been withdrawn from consideration. Claims 27-35 and 37-48 have been canceled without prejudice or disclaimer of any of the subject matter contained therein.

Rejection Under 35 USC §112, Second Paragraph

Claim 36 has been rejected under 35 USC §112, second paragraph as being indefinite. This rejection is respectfully traversed.

The Official Action asserts that the recitations "angiogenic phenotype" and "angiogenesis-inhibiting properties" claimed in claim 36 are ambiguous and indefinite. The Official Action asserts that it is unclear what "phenotype" and "properties" of the angiogenesis are contemplated.

Amended claim 36 now satisfies the provisions of 35 USC §112, since the phenotype of the endothelial cells is now clearly disclosed, i.e., the importance of the couple VEGF/VEGFR-2, and since the inhibiting properties of the antibody are drawn to the properties of the endothelial cells with an angiogenic phenotype.

Claim 36 is this clear, definite and has full antecedent basis.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Rejection Over MAYUMI et al.

Claim 36 has been rejected under 35 USC §102(b) as being anticipated by MAYUMI et al. This rejection is respectfully traversed.

MAYUMI et al. pertain to monoclonal antibody recognizing antigens expressed on the surface of tumor vessel endothelial cells.

MAYUMI et al. also pertain to a process for producing the monoclonal antibody. In particular, it is mentioned in MAYUMI et al. that the process comprises the steps of:

- immunization of animal with tumor endothelial cells, preferably with membrane fraction of said tumor endothelial cells,
- preparation of hybridomas, and
- checking inhibiting properties of obtained antibodies.

MAYUMI et al. is illustrated by the production of anti CD44 monoclonal antibodies, named TES antibodies, that inhibit cell proliferation of KTM-17 transplanted cells, i.e., by inhibiting the neovascularization of the KTM-17 derived tumor.

In particular, Example 1 of MAYUMI et al. sets forth a process for preparing monoclonal antibodies by pre-immunizing

mice with normal vessel endothelial cells (NVEC) membranes vesicles. NVEC cells disclosed in this document are purified and cultured as described in MADRI & WILLIAMS (cited at Column 9, lines 41-43 of MAYUMI et al., see attachment). Then, mice are immunized with tumoral endothelial cells derived from KTM-17 induced tumor.

MADRI & WILLIAMS pertain to a process for purifying and cultivating normal endothelial cells. These cells are cultured in an endothelial specific medium (medium 199E) supplemented with calf serum, antibiotics and glutamine (page 145, first column, end of second paragraph).

However, neither MAYUMI et al. nor MADRI & WILLIAMS disclose that endothelial need to be cultured with VEGF growth factor. Moreover, regarding the cell culture of endothelial cells (with angiogenic phenotype) this art never mentioned the use of VEGF for their proliferation or for their protection from apoptosis.

Regarding the tumoral endothelial cells, they are cultured in a medium formed from calf serum, antibiotics and glutamine. However, MAYUMI et al. (or MADRI & WILLIAMS) never mentioned that said cells are cultured with a supplement of growth factor, and *a fortiori* supplemented with VEGF.

Therefore, the process of the present invention is novel from MAYUMI et al., since the cells used in the present invention set forth specific characteristics that are not disclosed in MAYUMI et al.

MAYUMI et al. thus fail to disclose each and every element of claim 36 of the present invention. MAYUMI et al. accordingly fail to anticipate claim 36 of the present invention.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Rejection Over BURROWS et al.

Claim 36 has been rejected under 35 USC §102(b) as being anticipated by BURROWS et al. (*Clin. Cancer Research*, 1995 Dec; 1912): 1623-34). This rejection is respectfully traversed.

BURROWS et al. pertain to a monoclonal antibody directed against Endoglin, a protein present at the surface of endothelial cells. BURROWS et al. describe a process of producing this monoclonal antibody.

The process described in BURROWS et al. includes a step of immunizing mice with HUVEC endothelial cells, the HUVEC endothelial cells being previously stimulated with a HT-29 cells-conditioned medium. HT-29 cells are known to secrete angiogenic factor angiogenin (see reference FETT el al. *Biochemistry* 24: 5480-5486, 1985, cited in BURROWS et al.).

However, this art never mentions that endothelial cells (HUVEC) used in this process are ***strictly*** dependant upon VEGF for their growth and survival.

In contrast, the endothelial cells of the present invention are specifically dependent upon VEGF growth factor. This dependency confers specific features such as a high level

expression of VEGFR-2 receptor, where claim 36 sets forth that "said endothelial cells expression of VEGFR-2 is increased 4-fold in comparison with cells with a non-angiogenic phenotype."

The antibodies obtained by the process of the present invention are specific of endothelial cell with angiogenic phenotype, i.e., cells that form tubules in the presence of VEGF when they are cultured in collagen.

In contrast, endothelial cells such as HUVEC cells are commonly cultured in endothelial specific culture medium, with or without addition of growth factor. When growth factors are added, a culture medium for the *in vitro* maintenance of endothelial cells with angiogenic phenotype that contains "Endothelial cell growth supplement (ECGS)" (See ATCC enclosed data sheet of HUVEC – Document 1). This "supplement" is known in the art (MACIAG, PNAS, 1979, 76, 5674-78) for containing many growth factors, in particular Fibroblast Growth Factors (FGF-1, FGF2). BURROWS et al. would induce one to provide monoclonal antibody directed against tumoral endothelial cells by immunizing animals with endothelial cells activated by culture medium-secreted growth factors, i.e. mucin, TGFβ binding protein (See ATCC enclosed data sheet of I-1T9 cells – Document 2).

In comparison, the present invention selects new endothelial cells (dependent upon VEGF for their growth and survival), and these cells are used to produce antibodies specifically

inhibiting cells dependent upon VEGF, without affecting properties of endothelial cells without angiogenic phenotype.

In other words, the antibodies obtained by the process of the invention are not able to inhibits angiogenic properties of endothelial cells non-dependent upon VEGF for their growth and survival (cells disclosed in the cited prior art), but can inhibit cells that are liable to be activated upon VEGF stimulation, or that have been activated by VEGF.

BURROWS thus fails to teach each and every element of claim 36 of the present invention, such as "said endothelial cells form tubes in presence of growth factor VEGF." BURROWS therefore fails to anticipate claim 36 of the present invention.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Conclusion

The rejections are believed to be overcome, obviated or rendered moot, and that no issues remain. The Examiner is accordingly respectfully requested to place the application in condition for allowance and to issue a Notice of Allowability.

Prior art of record but not utilized is believed to be non-pertinent to the instant claims.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any

overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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APPENDIX:

The Appendix includes the following item(s):

- Verified English translation of foreign priority document No. 02/12606

- Madri et al., "Capillary Endothelial Cell Cultures: Phenotypic Modulation by Matrix Components", Journal of Cell Biology, Vol. 97, July 1983.

- Document 1 ATCC Search Catalog

- Document 2 ATCC Search Catalog